






Mallotus bullatus (Euphorbiaceae), a new species from Southwest China based on morphological characters and phylogenetic evidence

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Abstract

Mallotus bullatus M.T.An & J.H.Yu, **sp. nov.** (Euphorbiaceae), a species new to science discovered in Guizhou, China, is described and illustrated here, and its phylogenetic position among other *Mallotus* species is presented. Morphological, micro-morphological, and molecular evidence is presented as attestation of its novelty. The new species morphologically resembles *M. philippensis* var. *reticulatus* and *M. philippensis* var. *philippensis*, but it clearly differs by having bullate leaf surfaces (vs. not bullate), leaf margins entire or nearly so (vs. entire or nearly so in *M. philippensis* var. *philippensis* and coarsely serrate in *M. philippensis* var. *reticulatus*), leaf margins sometimes bearing red glands (vs. red glands absent), 5 sepals in staminate flowers (vs. 3–4 in *M. philippensis* var. *philippensis* and 4 in *M. philippensis* var. *reticulatus*), fruits with spines (vs. spines absent in *M. philippensis* var. *philippensis* and present in *M. philippensis* var. *reticulatus*), and abaxial leaf epidermal scattered and clustered vein hairs 0.1–0.8 mm long (vs. 0.04–0.28 mm long in *M. philippensis* var. *philippensis* and 0.05–0.1 mm long in *M. philippensis* var. *reticulatus*). Molecular phylogenetic analysis (BS = 100% / BS = 96%, PP = 1 / PP = 1) provides strong evidence supporting *M. bullatus* as a new species within the genus *Mallotus* and supports its placement in *M.* sect. *Philippinenses* as sister to *M. philippensis*.

Key words: Euphorbiaceae, Guizhou province, karst, molecular identification



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Introduction

Mallotus Lour. (Euphorbiaceae) is a large genus comprising approximately 150 species (Sierra et al. 2005), predominantly consisting of shrubs or trees, seldom climbers. It is mainly distributed in tropical and subtropical regions of Asia, Australia, and the Pacific, with a few species found in tropical Africa and Madagascar (Kulju et al. 2007a; Sierra et al. 2007). In China, there are approximately 30 species of *Mallotus*, mainly distributed in southern provinces and regions. The bark of some species is used for making ropes, and the seed oil

is used for soap and industrial oils (Liao and Liu 1958). Additionally, *Mallotus* species are important medicinal plants and sources of dye (Kumar et al. 2006; Sharma and Varma 2011; Dhaker and Sharma 2014). The genus *Mallotus* is an important component of forest vegetation (Slik et al. 2003; Eichhorn 2006), exhibiting a variety of life history strategies. Some species act as early successional pioneers, while others are climax species. The genus occurs in a wide range of habitats at low elevations (Sierra et al. 2007).

The genus *Mallotus* was established by De Loureiro in 1790 based on *Mallotus cochinchinensis* Lour. (Loureiro 1790). In the latest taxonomic studies of Euphorbiaceae (Webster 1994; Radcliffe-Smith 2001), the genus *Mallotus* is classified in the subtribe Rottlerinae Meisn. In addition, *Mallotus* has morphological, distribution and ecological similarities with *Macaranga* Thouars, another large genus in the Euphorbiaceae. Two phylogenetic studies (Slik and van Welzen 2001; Kulju et al. 2007b) specifically investigated the relationships of *Mallotus* with related genera. Kulju et al. (2007b) identified three clades, with the majority of *Mallotus* (*Mallotus* sensu stricto [s.str.]) forming a sister group to several small genera within the *Macaranga* clade. Sierra et al. (2010) compiled various datasets including plastid (matK) and nuclear (ITS) DNA sequences, macro-morphological characteristics, and leaf anatomical data, providing a detailed analysis of the phylogeny of *Mallotus*. The study revealed that *Mallotus* sect. *Mallotus*, sect. *Polyadenii* Pax & K.Hoffm., and sect. *Stylanthus* Pax & K.Hoffm. are monophyletic, while sect. *Axenfeldia* (Baill.) Pax & K.Hoffm. and sect. *Rottleropsis* Müll.Arg. are polyphyletic, and sect. *Philippinenses* Pax & K.Hoffm. is paraphyletic.

In 2023, during a botanical survey in the Maolan National Nature Reserve in Guizhou, China, we discovered a possibly new species of Euphorbiaceae. After more than a year of field investigations and specimen collection (GZAC-MU-0001), we conducted a field investigation on new species in Maolan National Nature Reserve, Guizhou Province, including photographing its characteristics and collecting seven live specimens. We found that its morphological characteristics resemble those of the genus *Mallotus*. To effectively distinguish the new species from other *Mallotus* species, this study utilized morphology, including pollen and leaf epidermal micromorphology, and molecular phylogenetics using ITS and matK sequences. The results led to the conclusion that the putative new species represents a new taxon.

Materials and methods

Morphology

Morphological features of leaves, inflorescences, flowers, and capsules were carefully observed and measured in the field, followed by detailed examination in the laboratory. Additionally, we compared specimens based on field observations and photographs taken, as well as studied related species using FAA-fixed materials and dried specimens (GZAC).

Leaf epidermis and pollen grains

The mature, complete pollen grains and leaves collected from the field were used to measure characters through a dissecting microscope. Subsequently,

they were mounted on stubs with double-sided tape, coated with a layer of gold, and then photographed using a Hitachi S-4800 scanning electron microscope. The micro-morphological characteristics of the pollen grains are described according to Wang and Wang (1983) and Nowicke and Takahashi (2002). The average size of the pollen grains is calculated based on 20 samples. The micromorphological features of the leaf epidermis of the genus *Mallotus* are stable genetic characteristics that show certain interspecific differences, reflect certain phylogenetic relationships, and can provide a basis for classification and species identification within the genus (Raju and Rao 1977; Alyas et al. 2020). Therefore, this study also investigates the leaf epidermal micromorphology of *Mallotus philippensis* H.Karst., including both var. *philippensis* and var. *reticulatus* (Dunn) F.P.Metcalf. The description of leaf micromorphological features follows Fiala et al. (1994) and Živa et al. (2012), and based on seven specimens collected in the field, including the holotype and the two paratypes.

Taxon sampling and DNA sequencing

We used a total of 36 species of *Mallotus* (Euphorbiaceae) in this study, including two individuals of the new species, and one outgroup species: *Macaranga trichocarpa* (Zoll.) Müll.Arg. We chose to use two molecular markers: ITS (ITS-1, 5.8S, and ITS-2) and matK. The ITS sequence, a highly reiterated tandem sequence in the nuclear genome, exhibits rapid changes, providing abundant variation and informative sites (Nürk et al. 2015) and a high level of species resolution accuracy (Chinese Plant Bol Group et al. 2011). The matK gene is one of the fastest-evolving genes in the chloroplast genome. It is easy to align and widely used in the study of families, genera and species (Khidir and Hongping 1997).

We extracted DNA sequences from fresh leaves of the new species and *M. philippensis* var. *reticulatus* using a modified CTAB protocol from Doyle and Doyle (1987), followed by PCR amplification and sequencing following the protocols in referred the methods of White et al. (1990) and Taberlet et al. (1991). We downloaded DNA sequences from GenBank for the two molecular markers for the remaining species used. Taxa and GenBank accession numbers are listed in Suppl. material 1: table S1.

Phylogenetic analysis

Sequences were aligned using default parameters in Clustal X v.1.83 (Thompson et al. 1997), followed by manual adjustments in BioEdit v.7.0 (Hall 1999). The phylogenies were constructed using Maximum Likelihood (ML) as implemented in PhyloSuite (Zhang et al. 2020) and Bayesian Inference (BI) as implemented in MrBayes v.3.0b4 (Ronquist and Huelsenbeck 2003), with the ITS and matK sequences analyzed separately. For the the nucleotide substitution model was chosen using the Akaike Information Criterion (AIC) in Modeltest v.3.06 (Posada and Crandall 1998), with the GTR+I+G model chosen for ITS and the GTR+I model for matK. For the ML analyses, the nucleotide substitution model was chosen using AIC in ModelFinder (in PhyloSuite), with the GTR+G model chosen for both regions.

Results

Taxonomic treatment

***Mallotus bullatus* M.T.An & J.H.Yu, sp. nov.**

urn:lsid:ipni.org:names:77351570-1

Fig. 1

Type. CHINA. • Guizhou Province, Libo County, Dawn township aquatic animals, 25°19'N, 107°56'E, alt. 700 m, 29 April 2024, *Ming-tai An, Jiang-hong Yu, Jian Xu, Feng Liu* GZAC-MU-001 (holotype GZAC!).

Diagnosis. *M. bullatus* can be distinguished from *M. philippensis* var. *reticulatus* and *M. philippensis* var. *philippensis* by having leaves with bullate surfaces and entire or nearly so margins, sometimes bearing red glands, 5 sepals in the staminate flowers, fruits with spines, pollen grains tricolporate with obvious furrows containing protrusions in the apertures, and abaxial leaf scattered and clustered vein hairs 0.1–0.8 mm long (Table 1).

Description. **Shrubs**, 1.5–2.5 m tall; twigs, young leaves, and inflorescences densely covered with yellowish-brown disc-shaped glandular hairs. **Leaves** simple, alternate, ovate or lanceolate, 5–18 (–22) × 3–6 cm, thickly papery, apex acuminate, base rounded or cuneate, margins entire or nearly so, sometimes bearing red glands, surface bullate, upper surface glabrous, lower surface densely grayish-yellow clustered-tomentose, with long soft solitary or clustered hairs on the veins, and scattered red disc-like glands; basal veins 3, lateral veins 3–4 pairs, looped and joined near the margin; extrafloral nectaries, 2–4, brown, near the base; petiole round 2–5 (–9) cm long, slightly pulvinate at both ends, covered with clustered hairs. **Inflorescences** racemose, terminal, solitary or clustered, solely staminate or pistillate, or mixed with pistillate flowers in lower part and staminate ones in upper part; sometimes apparently bisexual flowers also present. **Staminate inflorescences** 5–10 cm long, bracts ovate, ca. 1 mm long, pedicel 1–2 mm long, calyx lobes 5, oblong, ca. 2 mm long, densely covered with stellate hairs, with red disc-like glands; stamens 28–30. **Pistillate inflorescences** 3–8 cm long, bracts ovate, about 1 mm long; pedicels ca. 1–2 mm long; calyx lobes 4, ovate, densely covered with stellate hairs outside, ca. 3 mm long; ovary hairy, stigmas 3 split, 3–4 mm long, stigmas densely set with feather-like papillae on upper surface; some pistillate flowers sometimes bisexual, then with 1 or 2 stamens, the filaments almost as long as the anthers. **Bisexual inflorescences** 5–10 cm long, with 3–6 staminate flowers at the apex, lower part entirely pistillate; bracts ovate. **Capsule** subglobose, with spines, ca. 6–8 mm in diameter, fruit wall thickness ca. 1–2 mm, 3 carpellate, densely covered with red disc-like glands; **seeds** black, ovate or globose, naked with late mature stage.

Distribution and habitat. This species is known only from the karst landscape of Libo County, Guizhou Province, China (Fig. 2), alt. 700–900 m.

Phenology. Flowering from April to May, and fruiting from May to August.

Etymology. “Bullatus” specifically refers to the convex leaf areoles.

Local name. Simplified Chinese: 荔波野桐; Chinese Pinyin: lì bō yě tóng.

Leaf epidermis and palynology. Pollen grains of *M. bullatus* are spheroidal, with a size of 22 × 20 µm, L(long)/W(width) = 1.1, and tricolporate. They feature tricolporate furrows containing protrusions (Fig. 3A–C). Pollen grains of *M. philippensis* var. *reticulatus* are also spheroidal, 19 × 20 µm, L/W ratio

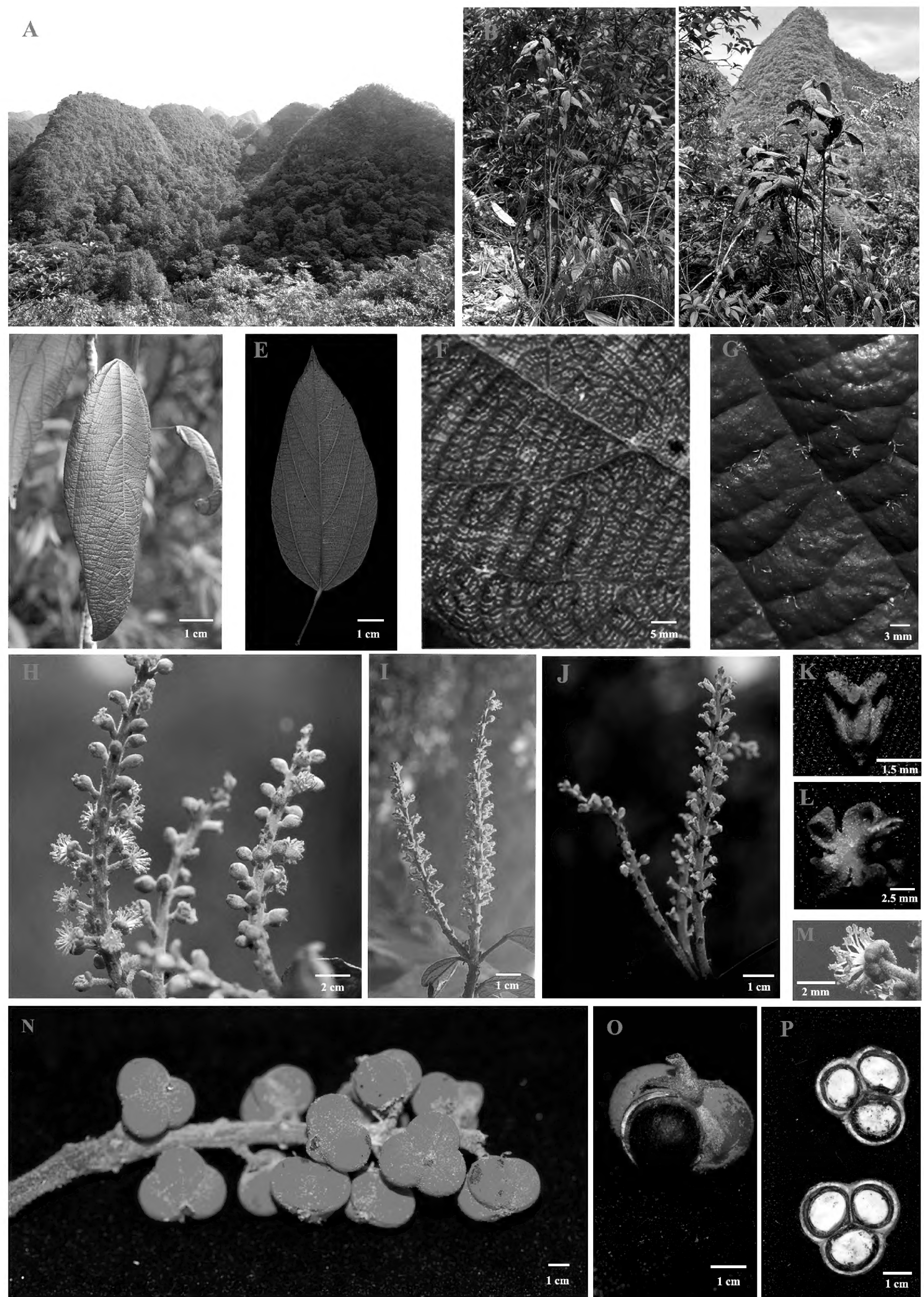


Figure 1. *Mallotus bullatus* **A** habitat **B, C** plant **D** leaf from above **E** leaf from below **F, G** leaf lower surface **H–J** inflorescences **K** pistillate flower **L, M** staminate flower sepals **N** infructescence with mature capsules **O** capsule with part removed to show seed **P** capsule in transverse section.

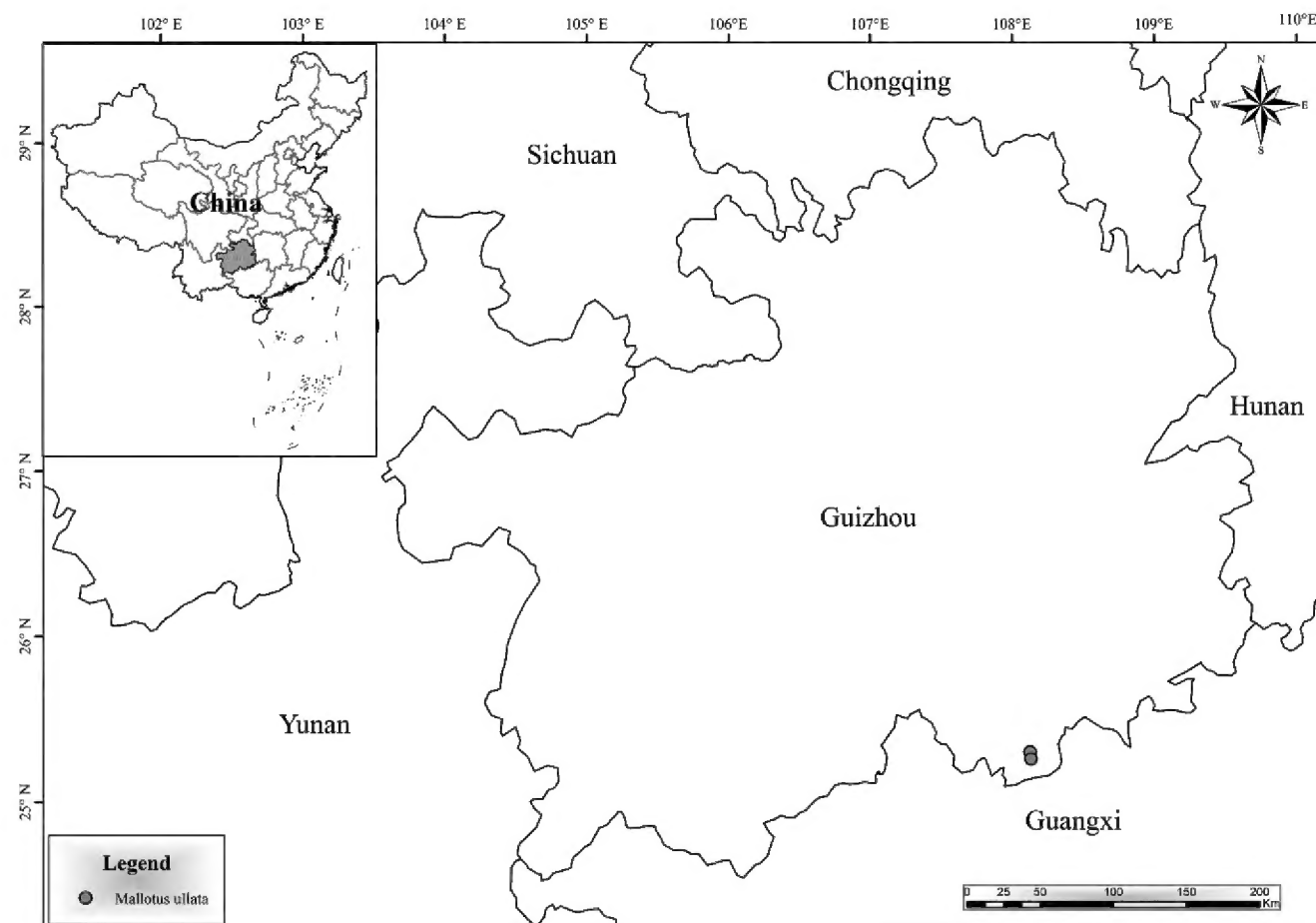


Figure 2. Geographical distribution of *Mallotus bullatus*.

0.95, without distinct furrows (Fig. 3D–F). The lower epidermis of *M. bullatus* leaves bears evenly distributed elliptical glands measuring $100 \times 70 \mu\text{m}$ ($E1 \times E2$: length of long equatorial axis \times length of short equatorial axis), is densely covered with short clustered hairs, and has long (0.1–0.8 mm long) solitary or clustered hairs on the veins (Fig. 3G, H). Similarly, the lower epidermis of *M. philippensis* var. *reticulatus* exhibits elliptical glands measuring $80 \times 70 \mu\text{m}$ ($E1 \times E2$), is densely covered with short clustered hairs, and has solitary or clustered hairs 0.05–0.15 mm long on the veins (Fig. 3J, K). The upper epidermis of both *M. bullatus* and *M. philippensis* var. *reticulatus* leaves is smooth (Fig. 3I, L).

Conservation status. During the period of 2023–2024, we sampled the population of *M. bullatus* and discovered two additional distribution points near the species initial discovery location (Fig. 2). Each site contained approximately 30 plants. The habitat of *M. bullatus* mainly occurs in karst scrublands, distributed from the foothills to the middle of the mountains. The plant habitat features poor soil fertility, low water retention capacity, and frequent drought conditions. Due to our current insufficient comprehensive assessment of the survival status and threats to *M. bullatus*, we cannot provide specific distribution information about this population. Therefore, we recommend categorizing *M. bullatus* as Data Deficient “DD” (IUCN 2022).

Morphological comparisons

Morphologically, the new species is similar to *M. philippensis* in having alternate leaves, basal veins 3, and racemose inflorescences. However, the new species can be distinguished from *M. philippensis* by its bullate leaf surface (vs. not bullate), leaf margins entire or nearly so (vs. entire or nearly so in *M. philippensis* var. *philippensis*, and coarsely serrate in *M. philippensis* var. *reticulatus*), leaf margins sometimes bearing red glands (vs. not red glands), fruits with spines (vs. absent in *M. philippensis* var. *philippensis* and present in *M. philippensis* var. *reticulatus*), 5 sepals in staminate flower (vs. 3–4 in *M. philippensis* var. *philippensis* and 4 in *M. philippensis* var. *reticulatus*) (Table 1, Suppl. material 1: fig. S1).

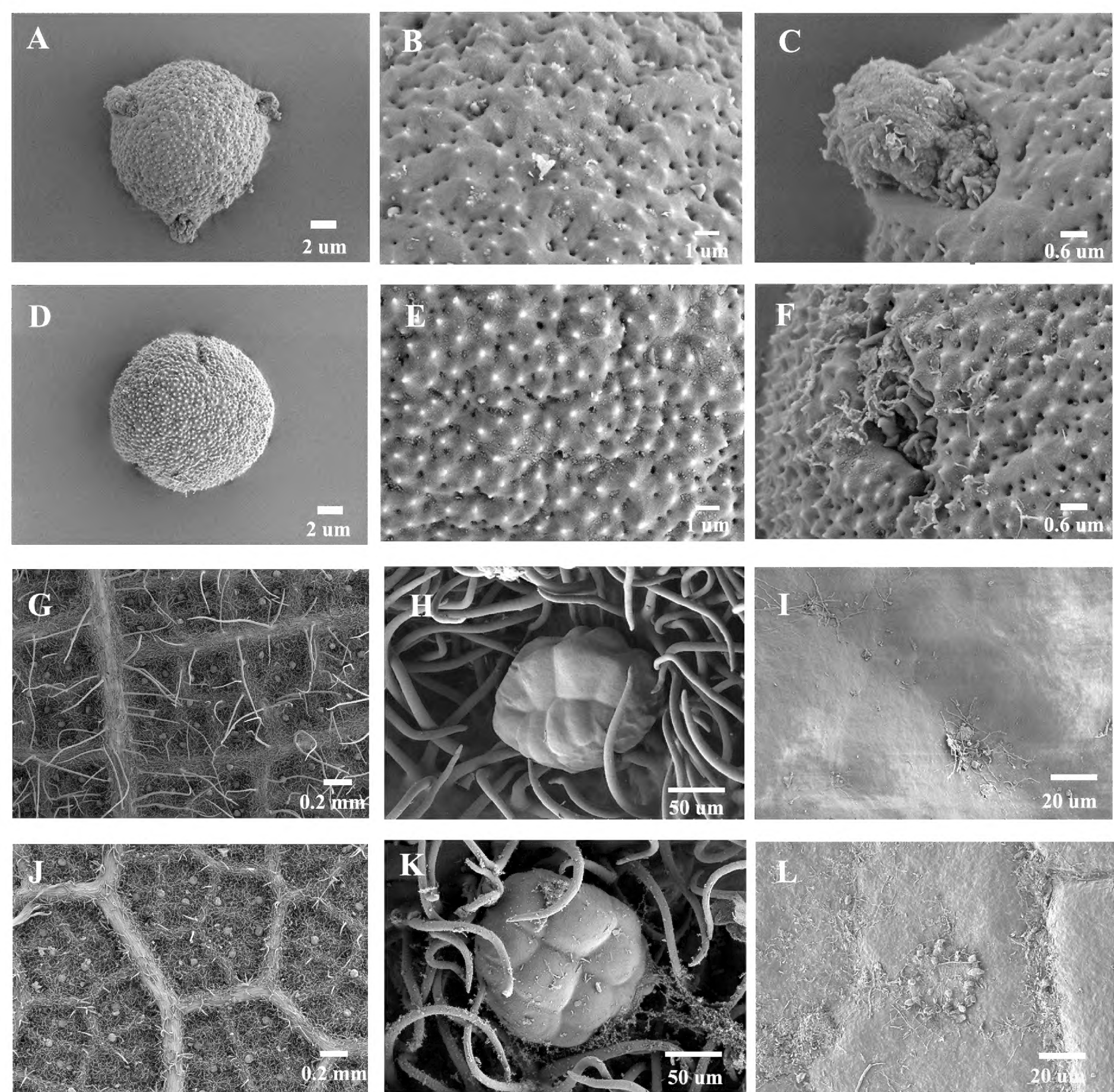


Figure 3. Scanning electron microscope images of *Mallotus* leaf epidermis and pollen grains **A–C** pollen grains of *M. bullatus* **D–F** pollen grains of *M. philippensis* var. *reticulatus* **G–I** lower leaf epidermis of *M. bullatus* **J–L** lower leaf epidermis of *M. philippensis* var. *reticulatus*.

Phylogenetic position

Nuclear data phylogenetic analyses

The length of the aligned ITS sequences of *M. bullatus* is 760 bp. Based on a dataset of 28 ITS sequences with 182 informative loci, phylogenetic relationships were analyzed using both Bayesian Inference (BI) and Maximum Likelihood (ML) methods (Fig. 4). The two sequences from the new species both originate from the same population, forming a strongly supported monophyletic clade (Fig. 4: BS = 100%, PP = 1). *Mallotus philippensis* var. *philippensis* and *M. philippensis* var. *reticulatus* are sister taxa with strong support (Fig. 4: BS = 100%, PP = 1), and they form a strongly supported sister group relationship with *M. bullatus* (Fig. 4: BS = 98%, PP = 1).

Table 1. Comparison of characteristic of three species of *M. bullatus*, *M. philippensis* var. *philippensis*, and *M. philippensis* var. *reticulatus*.

Character	<i>M. philippensis</i> var. <i>philippensis</i>	<i>M. philippensis</i> var. <i>reticulatus</i>	<i>M. bullatus</i>
Habit	Small tree or shrub	Shrub	Shrub
Upper leaf midrib hairys	No	Yes	No
Leaf margins	Entire or nearly so	Coarsely serrate	Entire or nearly so
Leaf margins bearing red glands	No	No	Sometimes
Leaf surface bullate	No	No	Yes
Length of solitary or clustered hairs on leaf abaxial veins	0.04-0.28 mm (Zhang, 2018)	0.05-0.1 mm	0.1-0.8 mm
Number of staminate sepals	3-4	4	5
Pollen size	15.5(17.5)-(11.6)15.5 µm (Nowicki and Takahashi 2002)	19 × 20 µm	22 × 20 µm
Fruits with spines	No	Yes	Yes

Plastid data phylogenetic analysis

The aligned matK sequences of *M. bullatus* are 2000 bp in length. Based on a dataset of 27 matK sequences with 557 informative loci, both Bayesian Inference (BI) and Maximum Likelihood (ML) analyses indicate that the two sequences from the new species form a strongly supported monophyletic clade (Fig. 5: BS = 96%, PP = 1). *Mallotus philippensis* var. *philippensis* and *M. philippensis* var. *reticulatus* forms a weakly supported sister clade (Fig. 5: BS = 80%, PP = 0.82), and they form a strongly supported sister group relationship with *M. bullatus* (Fig. 5: BS = 100%, PP = 1).

Discussion

According to the classification in Sierra et al. (2010), *M. bullatus* belongs to sect. *Philippinenses*, characterized by shrubby habit, alternate leaves, 3 basal leaf veins, and capsules densely covered with orange-red glands. Both phylogenetic trees (ITS and matK; Figs 4, 5) indicate that *M. bullatus* is a distinct member of the genus, and furthermore, support its sister group relationship with *M. philippensis* var. *philippensis* plus, *M. philippensis* var. *reticulatus*; these three species form a weakly supported clade with *M. repandus* (Figs 4, 5), also in sect. *Philippinenses*, thus corroborating the evidence provided by the morphological and micro-morphological observations. The two known populations of *M. bullatus* show no consistent morphological differences. Although *M. bullatus* forms a clade with *M. philippensis* var. *philippensis* and *M. philippensis* var. *reticulatus*, it differs from both taxa in its bullate leaf surfaces, length of hairs on the leaf abaxial veins, and number of sepals in the staminate flowers (Table 1).

Additional specimen examined

Mallotus philippensis var. *reticulatus* (Dunn) F. P. Metcalf. —CHINA. Fujian: Collected on Mr. Dunn’s expedition to Central, China. Apr. to Jun., 1905, 3429 (HH); West lake, Chenxiang town, Changtai district, Zhangzhou city. Jun. 11, 1976, Wang QJ, 012320 (AU). Jangxi: Yangling, Chongyi County, Ganzhou City, 24°29'N, 103°54'E alt. 1092 m, May 15, 2024, Yu JH, Tang YB, Wang YR, 20240501 (GZAC). *M. philippensis* var. *philippensis* (Lam.) Müll. Arg. — Yunan: roadside at the edge of forests, alt. 800 m. 2000, Shui YM, Chen WH 13773 (PE).

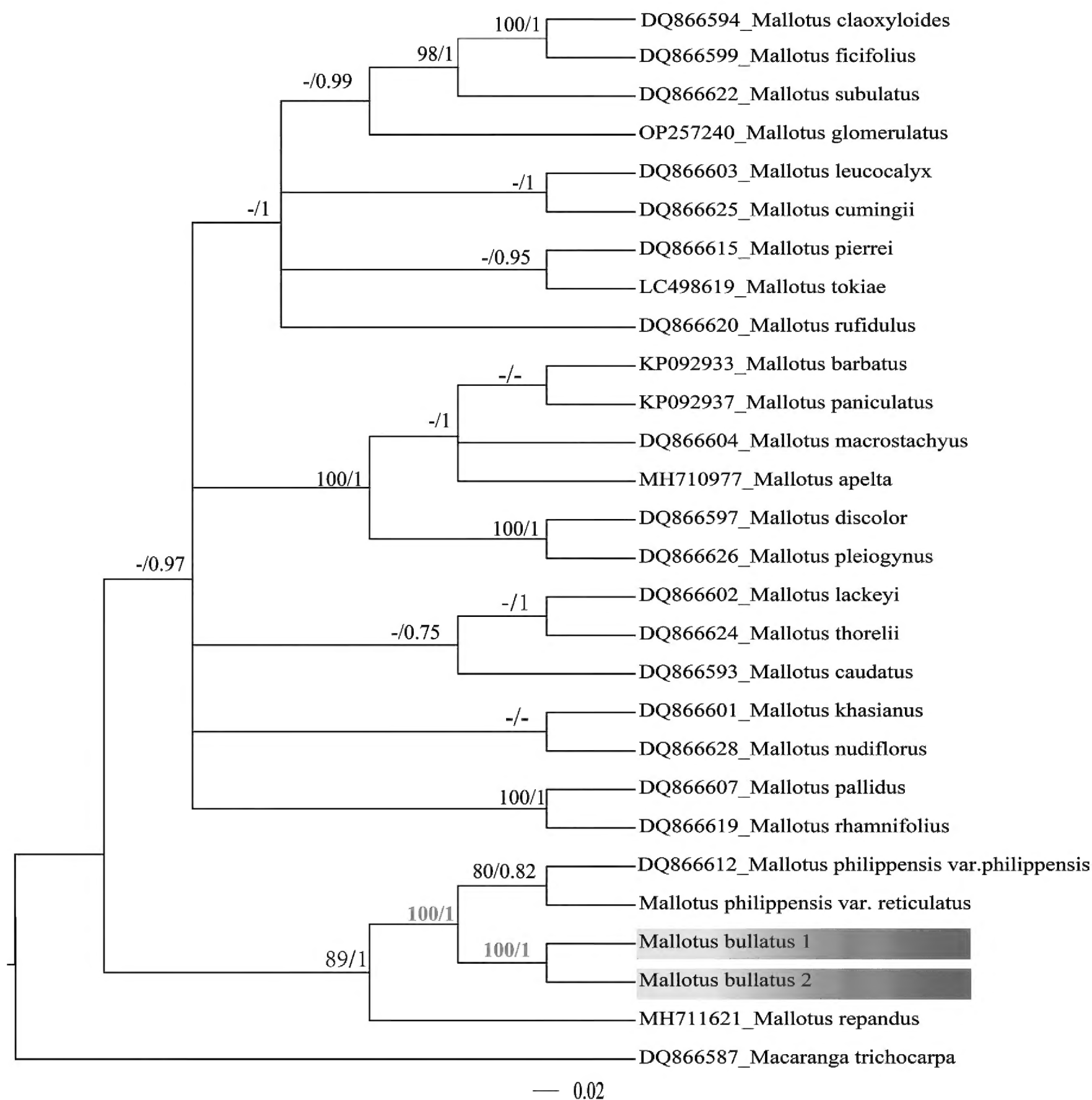


Figure 4. Partial Bayesian consensus phylogram based on ITS sequences. Numbers above branches are Bayesian posterior probabilities (PP) and Bootstrap probabilities (BS) (only PP values > 0.70, BS > 80 shown).

Sichuan: Jinjia Village, Sutie National Nature Reserve, Panzhihua, Sichuan Province, 26°37'29.7"N, 101°33'03.1"E, alt. 1653 m. Sept. 16, 2010. *Yang Y, Huang JH, Yang YQ, Liu B, Ye JF* 551 (PE). **Paratypes.** *Mallotus bullatus* — Guizhou: Rao gu village, Dawn township aquatic animals, Libo County, 25°19'N, 107°56'E, alt. 800 m, Apr. 29, 2024, *An MT, Yu JH, Xu J, Liu F* 202306-1 (GZAC); La nei village, Libo County, 28°21'N, 107°56'E, alt. 750 m, Apr. 30, 2024, *An MT, Yu JH, Xu J, Liu F* 202306-2 (GZAC). **Other specimens.** *Mallotus bullatus* — Guizhou: Yiba Mountain, Lane Village, Dawn township aquatic animals, Libo County, 25°16'N, 107°55'E, alt. 950 m, Jul. 26, 2024, *Yu JH, Tang YB, Liu F* 001 (GZAC); Yiba Mountain, Lane Village, Dawn township aquatic animals, Libo County, 25°16'N, 107°55'E, alt. 780 m, Jul. 28, 2024, *Yu JH, Tang YB, Liu F* 002 (GZAC); Dawn township aquatic animals, Libo County, 25°16'N, 107°57'E, alt. 840 m, Jul. 22, 2024, *Yu JH, Tang YB, Liu F* 003 (GZAC); Dawn township aquatic animals, Libo County, 25°16'N, 107°57'E, alt. 700 m, Jul. 22, 2024, *Yu JH, Tang YB, Liu F* 004 (GZAC).



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Conflict of interest

The authors have declared that no competing interests exist.

No ethical statement was reported.

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Author contributions

Jiang Hong Yu and Zheng Ren Chen completed all the work of this paper together, they contributed equally to this work and are the first co- authors of this paper. MingTai An planned and guided the writing of the whole paper, participated in the field investigation and identification work, acted as the corresponding author of the paper. Deng Li Yu participated in the field investigation and completed part of the data processing and content writing and guide the writing of the paper. Feng Liu Collect plant specimens in the field and take photographs for identification. Jian Xu participated in field investigation and compared plant specimens to identify species. Yu Bin Tang participated in field investigation and compared plant specimens to identify species. Yi Ran Wang participated in field investigation and data collation. Hua Kai Zou participated in field investigation and content writing.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Additional information

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Data type: docx

Explanation note: **table S1**. Taxa and GenBank accession numbers for thesequences used in this study. **fig. S1**. *Mallotus philippensis* var. *philippensis* (Lam.) Müll.Arg. and *M. philippensis* var. *reticulatus* (Dunn) F.P.Metcalf. **A, B** leaf of *Mallotus philippensis* var. *reticulatus* **C** holotype of *Mallotus philippensis* var. *philippensis* **D** holotype of *Mallotus philippensis* var. *reticulatus* **E** merotype of *Mallotus philippensis* var. *reticulatus*.

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